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(54) Title: PHARMACEUTICAL PREPARATION

(57) Abstract

A wound healing material comprising a water soluble compressed freeze-dried product containing a wound healing medicament and a base material can be placed directly on or into the wound. The material preferably contains human growth hormone as the medicament.

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PHARMACEUTICAL PREPARATION

DETAILED DESCRIPTION OF THIS INVENTION

This invention relates to water soluble compressed freeze-dried preparations containing a wound-healing medicament and a base material. Thus, this invention is directed to a pharmaceutical formulation designed for topical administration.

BACKGROUND OF THIS INVENTION

There are a variety of formulation types available for medicated topical therapy in wound management. There are solutions, powders, fluid suspensions or emulsions and semisolids. Semisolids such as creams, ointments, pasts and gels fulfil a special topical need in that they are able to cling to the application surface for an extended period, generally until they are washed out by the wound exudate or washed off by cleaning the wound area. Likewise, solutions have poor staying properties. Drugs or medicaments can be applied onto the wound by impregnation of or incorporation into dressings, swabs, films or gauze which are in contact with the wound tissue.

Freeze drying (also designated lyophilisation) is a well known process of drying. Lyophilisation of mixtures containing a medicament is known. The reasons for lyophilizing such mixtures are to obtain a stable preparation and to provide a long shelf life. The resulting product can be designated a lyophilisate.

The normal way of using such lyophilisates is to dissolve it in a solvent, normally water, prior to use. This procedure is called a reconstitution. The solution obtained constitutes the medicament which the patient is using like any other similar medicament.

An example of such a lyophilized formulation is described in European patent application having the publication No. 308,238. Example 7 therein describes a lyophilisate

containing hydroxypropylmethyl cellulose and epidermal growth factor.

It has for some time been recognized that a number of tropic factors, due to their stimulation of cell growth and differentiation, may have an enhancing effect on wound healing.

Several medicaments are known as having a wound healing effect. For example, human growth hormone (hereinafter designated hGH) being a superior pituitary gland hormone is known to have a wound healing effect. hGH can be administered topically. The effect of hGH on wound healing is thought to be mediated through growth factors. Specific growth hormone receptors on monocytes have been found. hGH may affect the monocytes to paracrine delivery of the well-known growth factors, i.e., PDGF, EGF, TGF-α and bFGF. Another explanation could be that hGH affects the fibroblasts to autocrine delivery of IGF-I. IGF-I has been shown to exhibit mitrogen effect on fibroblasts.

The object of this invention is to make available a medical formulation which conveniently can be used as a wound healing preparation.

DETAILED EXPLANATION OF THIS INVENTION

It has, surprisingly, been found that if a lyophilisate containing a wound healing medicament is compressed to a certain degree, a preparation which conveniently can be placed directly on or into the wound is obtained.

The lyophilisate is obtained by freeze drying a solution or suspension of a base material and a medicament having a wound healing effect. The content of medicament in the lyophilisate is conveniently in the range from about 0.1 - 10 percentage (weight/weight). Thereafter, the lyophilisate is compressed so as to obtain a soft and flexible product which, when placed on the wound, does not or only to a minor degree annoy the patient. Conveniently, the lyophilisate is compressed to from about 1/2 to about 1/10 of its original volume, preferably, to from about 1/3 to about 1/6.

The density of the compressed lyophilisates according to this invention is, preferably, in the range from about 50 to about 200 mg/cm^3, more preferred in the range from about 60 to about 120 mg/cm^3. The thickness of the compressed lyophilisates according to the invention is preferably in the range from 0.2 to 1.5 mm.

The base material present in the compressed lyophilisates according to this invention is conveniently a gelling agent.

An important aspect of the present invention is the use of an appropriate gelling agent in the dermatologically acceptable vehicle. The gelling agent used must be chemical inert in compositions with the medicament. Therefore, it must be non-ionic. Additionally, since the gel is to be prepared prior to lyophilization, the gelling agent must dissolve completely to produce a transparent gel. Additionally, the gelling agent used must allow for the hydrogel to release a sufficient amount of medicament within a few hours. Additionally, the gelling agent must form a hydrogel which allows for the generation of a coherent porous freeze-dried matrix by lyophilization.

Gelling agents possessing these qualities are specific cellulose ether compounds, preferably hydroxyethylcellulose, hydroxypropylcellulose hydroxypropylmethylcellulose, hydroxyethylmethylcellulose and methylcellulose, most preferred hydroxyethylcellulose (hereinafter designated HEC).

The material of this invention may be prepared by:

a) forming a liquid hydrogel containing the medicament dissolved therein, the viscosity of the hydrogel preferably being in the range from about 500 to about 10,000 cps, most preferred in the range from 1000 to about 4000 cps,

b) freeze drying the solution in a layer to form a freeze-dried matrix containing the medicament homogenously dispersed therein, and

c) applying pressure to the matrix to form a flexible sheet, where the medicament is in an effective dosage concentration per cm^2.

The wound healing materials of this invention are soft flexible sheets having a porous structure.

It is very convenient for the patient that the compressed lyophilisates can be placed directly on the wound. Hence, the patient does not have to make a reconstitution of the lyophilisate. In addition, it is possible to prepare compressed lyophilisates fron which pieces of a desired size can be cut or punched out to be placed on the wound.

It has been found that in the presence of moisture, some medicaments, for example hGH, looses biological activity during storage. Such loss of activity makes it impractical to store aqueous dermatological preparations containing such medicaments, for example hGH. This invention provides means for preventing loss of activity by providing stable lyophilized formulations containing the medicament means. This invention also provides means for preparing a composition comprising a gelling agent which is biological, chemical and physical compatible with the medicament.

Heretofore, hGH has not been lyophilized with a gelling agent and an extender to prepare a product suitable for use in wound healing application.

Another important aspect of the present invention is the use of polyethylene glycols (hereinafter designated PEG) in the vehicle. PEG acts as a bulk agent which imposes softness and smoothness to the freeze-dried sheet. PEG is dermatologically acceptable and chemically inert in compositions comprising various medicaments, for example human growth hormone. Additionally, PEG has only a minor effect on the viscosity of the hydrogel. PEG imposes softness to the freeze- dried matrix and prevents its surface from becoming icy and hard.

In order to stabilize some medicaments, for example hGH, buffer agents, cryoprotectants and a non-ionic surface active component are included in the hydrogel. Preferably, phosphate buffer (pH 7.0 = 7.5) Tween 80, mannitol and glycin are used.

The present invention describes a method to prepare soft and flexible sheets where both surfaces are smooth.

Another advantage of sheets prepared from the described method is their fast release of some medicaments, for example hGH. The sheet forms a hydrogel initially after contact with the aqueous medium and releases up to 100% of some medicaments, for example hGH, into the surrounding aqueous medium.

The flexible sheet of the invention can be combined with a backing material being a wound dressing or bandage which also forms an aspect of the invention. Most preferred, the medicated sheet sticks to an adhesive dressing producing an integrated medicated wound cover. The sheet may be combined with a hydrocolloid dressing comprising the same hydrocolloid as the material in the sheet. The hydrocolloid in the dressing as well as in the sheet swells locally in a linear fashion and expands into the wound cavity where it applies both osmotic and physical pressure to the wound bed.

The freeze-dried sheet offers a potential for combined products with hydrocolloid dressings or films where the sheet sticks to the dressing or film. Wound dressing is here used to embrace a wide spectrum of materials and includes absorbents, surgical adhesive tapes and bandages.

The invention is explained with reference to the drawings on which

Fig. 1 shows a Scanning Electron Microscopy of a lyophilized product according to the invention, and

Fig. 2 shows a Scanning Electron Microscopy as in Fig. 1 but in a greater scale.

The invention is further explained in the below examples which are presented to illustrate this invention. This invention is not to be considered limited by these examples.

EXAMPLE 1

A preferred method to prepare a smooth, soft and flexible dry sheet is as follows: A solution containing 1.0% PEG 6000 and 1.5% hydroxyethylcellulose (Natrosol Hx Pharm 250 TM) was prepared in water. B-hGH (biosynthetic hGH) was added result-

ing in a final concentration of 200 µg of per gram gel. The gel was placed in 5 cm diameter petri-dishes in a layer of 3 mm.

The gel was lyophilized. The dry gel was soft and homogenous. It was pressed into thin (1 mm) soft sheets and cut into pieces of appropriate sizes. In an in vitro kinetic study in tris-buffer (pH=7.4), the sheet was found to dissolve and release 100% of its hGH during 0.5 hours (37°C).

The hydrogel prepared above was placed in 7.5 ml vials in a layer of 3 mm. The vials were placed in a lyophilization chamber and the following freeze-drying cyclus was used:

Step 1) -40°C for 4 hours at 1 atm.
Step 2) -40°C for 1 hour at full vacuum.
Step 3) -30°C for 3/4 hour at full vacuum.
Step 4) -20°C for 4 hours at full vacuum.
Step 5) 0°C for 15 hours at full vacuum.
Step 6) 30°C for 11 hours at full vacuum.

The vials were closed in the freeze-dryer at the end of the cyclus. Samples were analyzed for hGH and its polymer and dimer forms and for content of moisture.

Batch	hGH, mg/g	polymer, %	dimer, %	H_2O, %
91120 t = 1 month (4°C)	18	0.7	4.2	-
909-18 t = 0	18	0.7	6.0	2.0

EXAMPLE 2

In order to study the stability of dry gels containing hGH, gels were freeze-dried in 7.5 ml glass vials in a layer of 3 mm as described in Example 1.

The following compositions were prepared for accelerated stability study:

Composition	a	b	c	Control	
hGH (IU)	5	5	5	5	IU
Glycin	1.3	1.3	1.3	1.3	mg
Mannitol				36	mg
$NaH_2PO_4 \cdot H_2O$	.7	.7	.7	.7	mg
$Na_2HPO_4 \cdot 12H_2O$	5.3	5.3	5.3	5.3	mg
PEG 6000			8.0		mg
Tween 80		2.0	2.0		mg
Natrosol Hx250	24	24	24		mg

The vials were stored at 25°C and 37°C for 2, 4 and 8 weeks.

The results are shown in the below Table.

TIME	TEMP.	COMPOSITION	hGH IU	DESAMIDO %	POLYMER %	DIMER %	H_2O %
0	-	a	4.6	4.5	<0.5	1.4	3.5
		b	4.8	4.4	<0.5	2.0	3.1
		c	5.2	4.5	<0.5	-	2.8
		control	5.6	4.2	0.7	2.3	1.3
2 weeks	25 C	a	5.0	3.9	0.5	2.3	
		b	4.2	4.0	<0.5	2.2	
		c	4.2	3.6	<0.5	2.9	
		control	5.8	3.9	0.6	1.5	
	37 C	a	4.4	4.8	<0.5	3.5	
		b	4.8	4.7	<0.5	3.1	
		c	4.6	4.2	<0.5	4.1	
		control	5.4	3.5	0.8	5.7	
4 weeks	25 C	a	-	5.0	0.7	2.3	
		b	-	3.4	<0.5	2.2	
		c	-	3.6	<0.5	2.9	
		control	-	1.7	0.5	1.5	
	37 C	a	4.6	6.4	<0.5	3.9	
		b	4.2	4.1	<0.5	4.1	
		c	4.6	5.3	<0.5	6.1	
		control	4.2	2.9	0.8	8.0	
8 weeks	25 C	a	-	3.4	1.0	2.2	
		b	-	3.8	<0.5	2.6	
		c	-	3.6	<0.5	2.8	
		control	-	3.4	1.5	2.7	
	37 C	a	4.8	7.9	<0.5	3.6	
		b	4.8	6.1	<0.5	4.7	
		c	4.6	6.5	<0.5	6.3	
		control	4.6	6.6	0.8	8.8	

The data show that the dry gels containing hGH are stable and that hGH in the freeze-dried gels has a stability similar to that of the control which is a conventional composition for parenteral use.

EXAMPLE 3

This example illustrates that hydroxyethyl cellulose is compatible with hGH for the purpose of the present invention.

The below preparation containing hGH and 0.6% hydroxyethylcellulose in water was tested for biological activity by the Tibia test in rats.

A hydrogel consisting of the following components was prepared:

hGH (Norditropin^{TM}), 4 IU/ml:	1000 µl and 500 µl.
sodium chloride solution (0.9%):	9.00 ml.
hydroxyethylcellulose (Natrosol Hx Pharm 250), 1.2%:	10.00 ml.

According to the result from the tibia test, the biological activity of hGH in this hydrogel was maintained.

An in vitro hGH-receptor assay was performed by comparing an aqueous composition containing 1.2% hydroxyethylcellulose with a control composition without the gelling agent. The cell used was IM-9 human cell line. The assay is based on competition between a known labelled hGH (^{125}I-hGH) and the unknown hGH sample. The measured radioactivity is then related to a constructed standard curve.

1. hGH: 12 IU/ml in water (control).
2. hGH: 12 IU/ml in 1.2% Natrosol Hx Pharm in water.

There was no significant difference in activity between the two compositions.

EXAMPLE 4

In this example physical properties of two compressed dry gel products are characterized.

Dry gels were prepared by freeze drying hydrogel solutions of the following compositions:

	A	B	
hGH	.05	.05	%
Natrosol HX250 Pharm.	1.5	1.5	%
PEG 6000	1.0	1.0	%
Glycin		0.5	%
Tween 80		.5	%
Mannitol		.5	%
$Na_2HPO_4.2H_2O$		.19	%
$NaH_2PO_4.H_2O$		.037	%
Sterile water ad	100	100	%

27 g hydrogel solution were filled into 9 cm petri dishes and lyophilized.

The derived dry gels had a hight of 4 mm. They were punsched into dics of 10 mm diameter.

Test of tensile strength was performed according to DIN 53455:

Gel type A: 0.093 N/mm² B: 0.095 N/mm²

Test of pressure resistance was performed according to DIN 534553:

Gel type A reduction of hight (%)	Gel type B reduction of hight (%)	force applied (N/mm²)
5	7.5	.005
8.8	18	.01
20	49	.02
43	65	.03
58	71	.04

Scanning Electron Microscopy of the compressed freeze-dried product prepared from gel type A was performed. A cross-section of a disc was examined and the porous structure of the sheet is clearly shown by ESM-pictures in Figures 1 and 2.

CLAIMS

1. Wound healing material, characterized in that it comprises a water soluble compressed freeze-dried product containing a wound healing medicament and a base material.

2. Material according to claim 1 characterized in that the medicament is human growth hormone.

3. Material according to claim 1 or 2 characterized in that the base material is a hydroxycellulose ether, preferably hydroxyethylcellulose, hydroxymethylpropylcellulose hydroxypropylcellulose, methylcellulose or hydroxyethylmethylcellulose, preferably hydroxyethylcellulose.

4. Material according to any one of the preceding claims characterized in that it contains PEG having a molecular weight in the range from about 3000 to about 20,000, preferably from about 6000 to about 8000.

5. Material according to any one of the preceding claims characterized in that it contains a buffer agent.

6. Material according to any one of the preceding claims characterized in that it contains a non-ionic surface active component and optionally a water soluble extender for lyophilization.

7. Material according to any one of the preceding claims, characterized in that the concentration of human growth hormone is in the range from about 0.1 to about 2.0 IU/cm^2.

8. Material according to any one of the preceding claims, characterized in that the concentration of hydroxycellulose ether is in the range from about 20 to about 90%, preferably from about 30 to about 75% (weight/weight).

9. Material according to any one of the preceding claims, characterized in that the concentration of PEG is in the range from about 0 to about 70%, preferably from about 15 to about 50% (weight/weight).

10. Material according to any one of the preceding claims, characterized in that the flexible sheet is porous and has a density larger than about 0.05 g/cm^3.

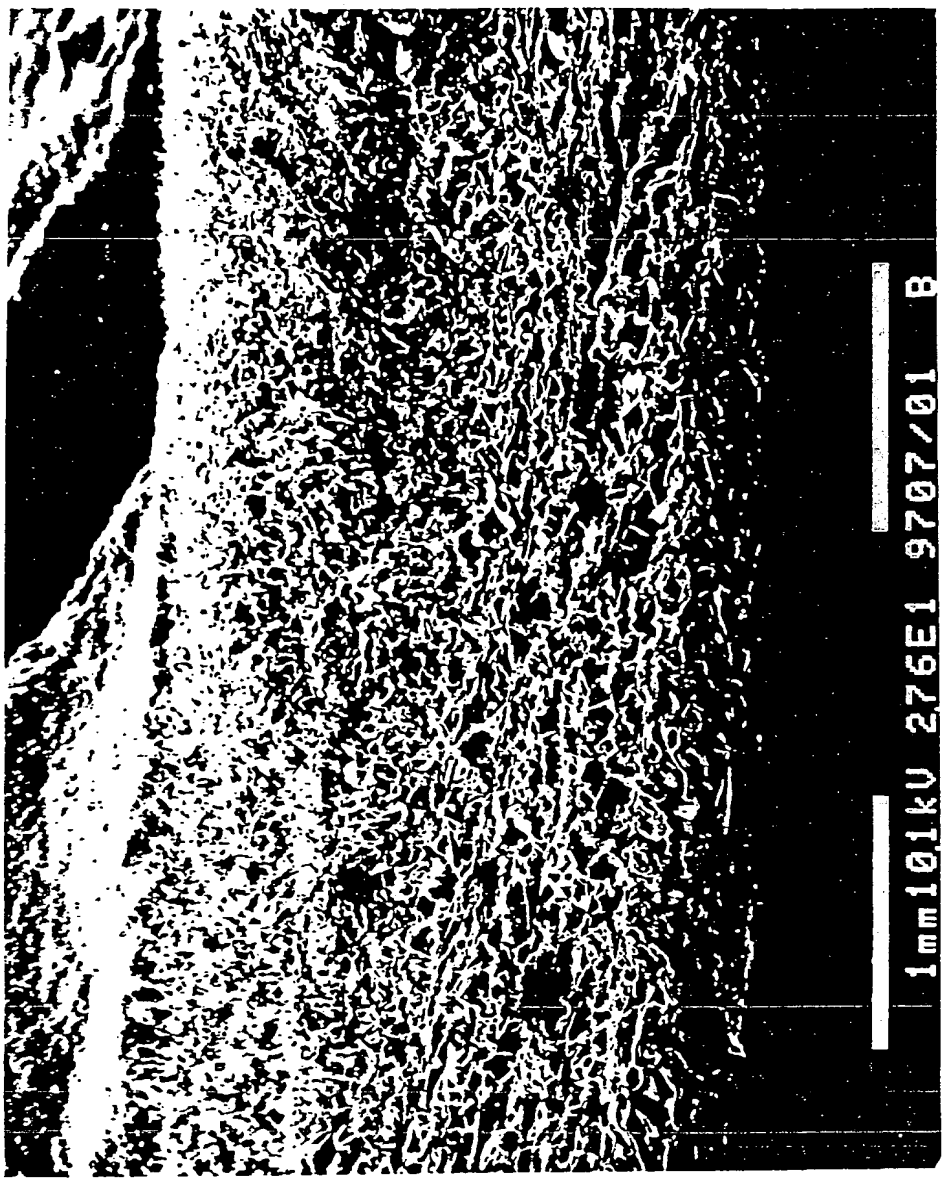

FIG. 1

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 91/00156

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 9/06

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 61 K; C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	EP, A1, 0308238 (ETHICON INC.) 22 March 1989, see the whole document --	1-10
Y	EP, A2, 0339905 (ETHICON INC.) 2 November 1989, see the whole document --	1-10
Y	EP, A2, 0261599 (EXOVIR, INC.) 30 March 1988, see the whole document --	1-10

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* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24th September 1991	1991 -09- 30
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Anneli Jönsson

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/DK 91/00156

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 91-08-30
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A1- 0308238	89-03-22	AU-D-	2223688	89-03-23
		JP-A-	1121223	89-05-12
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